Symposium: Myosin Binding Protein-C: A Modulator of Cardiac Contractility

3038-Symp
Modulating Contraction by Binding of MyBP-C to Actin
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Myosin-binding protein C (MyBP-C) is a ~130 kDa protein of the thick filaments of vertebrate skeletal and cardiac muscle. It consists of a linear array of ten or eleven globular, 10-kDa domains from the immunoglobulin (Ig) and fibronectin type III families, and an additional, MyBP-C-specific motif. The cardiac isoform, cMyBP-C, plays a key role in modulating cardiac function, and mutations in MyBP-C cause heart disease. Despite its discovery 40 years ago, the mechanism of MyBP-C function remains poorly understood. *In vitro* studies suggest that it could modulate contraction by binding to thin filaments, but there has been no evidence for this *in situ*. We used electron tomography of exceptionally well-preserved skeletal muscle to study the 3D organization of MyBP-C in the intact sarcomere. The tomogram shows that MyBP-C projects perpendicular to the thick filament surface and reaches neighboring thin filaments. This thick-thin filament bridge suggests a possible physical basis for modulating filament sliding and thus contraction. *In vitro*, binding to actin has been shown to occur via MyBP-C's N-terminal end. To understand the structural basis of this binding, we used negative stain electron microscopy and 3D reconstruction to study F-actin decorated with bacterially expressed N-terminal cMyBP-C fragments. Clear decoration was obtained under a variety of salt conditions. 3D reconstructions showed MyBP-C density starting over subdomain 1 of actin and extending tangentially towards actin's pointed end. Molecular fitting with an atomic structure of a MyBP-C Ig domain suggested that most of the N-terminal domains may be well ordered on actin. The location of binding was such that it appeared to overlap the relaxed (low Ca^{2+}) position of tropomyosin but not the activated position. This suggests that MyBP-C might help determine the state of thin filament activity by modulating tropomyosin position on actin.

3039-Symp
Dynamic Regulation of Contraction by Cardiac Myosin Binding Protein-C
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Cardiac myosin binding protein-C (cMyBP-C) is a thick filament associated protein that performs both regulatory and structural roles within cardiac sarcomeres. It is a member of the immunoglobulin (Ig) superfamily of proteins consisting of 8 Ig- and 3 fibronectin (FNIII)-like subdomains along with a unique regulatory sequence referred to as the M-domain whose structure is unknown. Here we used atomic force microscopy (AFM) to probe the structure and mechanical properties of the different subdomains of native and recombinantly expressed cMyBP-C molecules. Results demonstrate that cMyBP-C exhibits complex mechanical behavior under load and contains multiple domains with distinct mechanical properties. The Ig and FNIII-like domains unfold over a range of relatively low forces (50-190 pN), whereas the M-domain is readily extensible at forces $<$50 pN and is likely to be an intrinsically disordered segment of cMyBP-C. Additional extensible segments of cMyBP-C are likely to include linkers between the Ig domains such as the proline-alanine rich sequence between domains C0 and C1. Taken together these results suggest that cMyBP-C is compliant and readily extensible, potentially conferring structural and functional plasticity to cMyBP-C during the course of a single heart beat. Supported by NIH HL080367.

3040-Symp
Cardiac Myosin Binding Protein-C Phosphorylation, Contractile Function and Cardioprotection
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Cardiac myosin binding protein-C (cMyBP-C) is a sarcomeric thick filament assembly protein with regulatory functions in the heart. The cMyBP-C protein differs from the skeletal isoform in that it has a small insertion near the carboxyl terminus that contains 3 phosphorylatable serines, Ser-273, −282 and −302. While the precise functional correlates of cMyBP-C phosphorylation remain obscure, we do know that cMyBP-C is targeted by multiple kinases, such as PKA, PKC, RSK, PKD, CaMKII and PKG, suggesting that it plays a vital role in cardiac signaling. We previously reported that cMyBP-C phosphorylation is essential for normal heart function and that Ser-282 phosphorylation is critical for the subsequent phosphorylation of Ser-302 and normal cardiac function. However, the role of Ser-282 cMyBP-C phosphorylation in cardiac function, particularly as it affects contractile properties and sarcomere organization, is unclear. Therefore, to better understand the mechanisms and significance of cMyBP-C phosphorylation, we established several transgenic mouse models to determine the necessity and sufficiency of Ser-282 phosphorylation for normal cardiac function. Our findings suggested that cMyBP-C phosphorylation at Ser-282 is essential for normal cardiac function and that dephosphorylation at this site accelerates cMyBP-C degradation and cleavage of a 40 kDa fragment. During MI, we showed that cMyBP-C is extensively fragmented when dephosphorylated and that such fragmentation correlates well with contractile dysfunction and heart failure. Meanwhile, we also established that the release of cMyBP-C in the blood post-MI could be a potential diagnostic biomarker for MI. Overall, these studies show that Ser-282 phosphorylation is a critical determinant of Ser-302 phosphorylation and that cMyBP-C dephosphorylation accelerates its degradation and release into the circulation. In conclusion, we provide strong evidence that cMyBP-C phosphorylation directly affects the heart's contractile properties, sarcomere organization and cardioprotection.

3041-Symp
Cardiac Myosin Binding Protein C Phosphorylation in Human Cardiac Disease
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During recent years it has become increasingly evident that cardiac myosin binding protein C (cMyBP-C) exerts an important role in regulation of sarcomere function with consequences for *in vivo* cardiac performance. The functional role of cMyBP-C is tightly regulated by kinase-mediated phosphorylation. The most important kinase which phosphorylates cMyBP-C *in vivo* is protein kinase A (PKA), which is activated upon stimulation of the β-adrenergic receptors during exercise. In end-stage failing human myocardium, reduced phosphorylation of cMyBP-C has been reported using 1-dimensional and 2-dimensional gel electrophoresis. This reduced phosphorylation has been attributed to down-regulation and desensitization of the β-adrenergic receptor pathway in end-stage human heart failure. Low levels of cMyBP-C phosphorylation were also found in patients with familial hypertrophic cardiomyopathy (FHC), which is frequently caused by mutations in genes encoding sarcomeric proteins, with one exception: in FHC patients with mutations in the gene which encodes cMyBP-C (*MYBPC3*), phosphorylation of cMyBP-C was unaltered compared to non-failing donor hearts. cMyBP-C can be phosphorylated *in vivo* on at least three serine sites (Ser273, Ser282 and Ser 302), all of which are located in the cardiac isoform specific M region. At least one other site should exist in humans. With tandem mass spectrometry we recently identified a fourth phosphorylation site on Ser133, which is present in the Pro-Ala rich region that links the C0 and C1 domains. Preliminary data indicate that Ser133 is not phosphorylated by PKA. Analysis of phosphorylation on Ser133 in failing and donor samples revealed lower levels of Ser133 phosphorylation in several forms of cardiac disease compared to non-failing myocardium. Overall, our studies indicate that diverse cMyBP-C phosphorylation patterns exist in human cardiomyopathies, which may in part underlie sarcomere dysfunction observed in human heart failure.

Symposium: Materials Science Meets Biology

3042-Symp
Engineering Cooperative Nanosytems
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Our laboratory is interested in engineering tools and systems using nanoparticle scaffolds to transform the diagnosis and treatment of cancer. We aim to integrate nanomaterials with enhanced nanoscale properties and bioresponsive functionalities with our knowledge of the tumor microenvironment to explore this paradigm. Towards this end, we have developed and investigated nanoparticle conjugates based on three nanoparticle cores that harness features of the nanoscale: semiconductor quantum dots that exhibit size-based optical properties, dextran-coated iron oxide particles whose assembly alters the spin-spin relaxation time of hydrogen protons on magnetic resonance imaging, and polymer-coated gold nanorods that interact resonantly with near-infrared light. Our studies have shown how these nanoparticles specifically designed to

enhance their interaction with the biological environment can help achieve targeting, triggered self-assembly, remote actuation with radiofrequency fields, sensing of kinase activity, and delivery of short interfering RNAs. In collaboration with Erkki Ruoslahti (Burnham Institute), we have explored how decorating the surface with peptides obtained from in vivo phage display can alter the properties of these nanoparticles and control their trafficking. To increase the accumulation of the nanoparticles at the tumor site we are exploring in vivo self-assembly of these particles. Our approach is inspired by platelets_ natural microparticles that normally circulate in a latent form but can home to sites of injury and transform to an activated state, whereby they adhere and recruit more platelets. This results in assemblies of magnetic nanoparticles that may then acquire emergent properties, allowing either their enhanced visualization or remote actuation of drug delivery. More recently, we have also emulated biological systems where biological components remotely communicate via biological intermediates. The resultant nanoparticle formulations then act as a ''system'' to produce emergent behaviors for enhancing diagnosis and therapy.

3043-Symp

Co-Opting Moore's Law: Vaccines and Medicines made from a Wafer

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In 1965, Gordon Moore, co-founder of Intel, described the trend that the number of components in integrated circuits had doubled every year since 1958. This trend has continued to today, enabled by advances in photolithography which has taken the minimum feature size of transistors down from about 10 microns in 1970 to 0.045 microns (45 nm) today. In biological terms, this corresponds to going from the size of a red blood cell to the size of a single virus particle! As such, this top-down nano-fabrication technology from the semiconductor industry is, for the first time, in the size range to be relevant for the design of medicines, vaccines and interfacially active Janus particles. This lecture will describe the design, synthesis and efficacy of organic nano- and micro-particles using a top-down nano-fabrication technique we developed called PRINT (Particle Replication in Non-wetting Templates). PRINT is a continuous, roll-to-roll, high resolution molding technique that allows the fabrication of precisely defined micro- and nano-particles in a continuous manner with control over chemical composition, size, shape, deformability and surface chemistry. With these 'nanotools', we are establishing definitive biodistribution maps to elucidate the interdependent roles that size, shape, deformability and surface chemistry play on particle distribution as a function of different dosage forms (IV, IP, inhaled, subcutaneous, intramuscular, etc). This information is setting the stage for the design of highly effective chemo-therapeutics, chemo-preventions and cancer vaccines which will be described.

3044-Symp

Studies in the Three-Dimensional World of a Cancer Community of Cells

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Cancer is a dynamic condition in a complex community of cells which move and communicate in 3-D space and complex topologies. We will present results using 3-D microfabrication and materials science which maps out how the community moves and invades.

3045-Symp

Engineering of Polymeric Nanoparticles from Medical Applications

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A variety of organic and inorganic materials have been utilized to generate nanoparticles for drug delivery applications, including polymeric nanoparticles, dendrimers, nanoshells, liposomes, nucleic acid based nanoparticles, magnetic nanoparticles, and virus nanoparticles. The two most commonly used systems are polymeric nanoparticles and liposomes. Controlled release polymer technology has impacted virtually every branch of medicine, including ophthalmology, pulmonary, pain medicine, endocrinology, cardiology, orthopedics, immunology, neurology and dentistry, with several of these systems in clinical practice today such as Atridox, Lupron Depot, Gliadel, Zoladex, Trelstart Depot, Risperidol Consta and Sandostatin LAR. The annual worldwide market of controlled release polymer systems which extends beyond drug delivery is now estimated at $100 billion and these systems are used by over 100 million people each year. Polymeric nanoparticles can deliver drugs in the optimum dosage over time, thus increasing the efficacy of the drug, maximizing patient compliance and enhancing the ability to use highly toxic, poorly soluble, or relatively unstable drugs. These systems can also be used to co-deliver two or more drugs for combination therapy. The surface engineering of these nanoparticles may yield them ''stealth'' to prolong their residence in blood and the functionalization of these particles with targeting ligands can differentially target their delivery or uptake by a subset of cells, further increasing their specificity and efficacy. More recently combinatorial approaches have been developed to precisely engineer nanoparticles and screen multiple nanoparticle characteristics simultaneously with the goal of identifying formulations with the desired physical and biochemical properties for each specific application. The goal of this talk is to review our efforts in the design and optimization of polymeric nanoparticles for medical applications, which formed the foundation for the clinical translation of the first-in-human targeted and controlled-release nanoparticles (BIND-014) for cancer therapy.

Platform: Emerging Single Molecule Techniques

3046-Plat

Fast Three-Dimensional Imaging and Tracking of Single Molecules in the Nucleus of Live Cells using a Multifocal Microscope

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Deciphering the dynamic nature of biological processes requires developing tools for monitoring the behavior of single molecules (SMs) in live cells. However, a key challenge lies in the difficulty of imaging and tracking SM in three dimensions, notably for intracellular proteins that tend to diffuse rapidly ($>$ 0.5 um2/s). Methods currently employed for sub-diffraction 3D localization of individual fluorophores are limited to an axial depth of ~1 um or less. Here we present a novel multifocal microscope that permits the rapid (up to 30 frames/s) acquisition of 3D stacks. The microscope is based on the incorporation in the emission pathway of a multi-focus diffraction grating combined with a chromatic-correction grating in order to simultaneously detect 9 different axial planes on a single detector camera. Thereby, the system allows for aberration-free detection of individual molecules over an axial distance of up to 5 um. The microscope characteristics are particularly appropriate for probing the dynamics of individual molecules in the nucleus of cultured cells. This capability is demonstrated by recording the 3D motion of individual inert markers (beads or quantum dots), mRNAs, histone H2B proteins or transcription factors in live mammalian cells. Overall, our multifocal microscope, with its ease of use and performances, holds great promise for fast and sensitive 3D imaging in many cellular contexts.

3047-Plat

Fluorescence Triple Correlation Spectroscopy Resolves Ten Intermediates Along Different Parallel Ribosome Assembly Pathways

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Efficient self-assembly of the bacterial 30S ribosomal subunit depends on the interplay of a large number of sequential RNA folding and protein binding events. In many cases the order of these events is not rigidly defined, giving rise to an assembly landscape crossed by parallel assembly pathways. The biological and biophysical implications of parallel assembly are unclear, and seem to contradict the current understanding of the ways large RNAs avoid kinetic traps. There has been very little direct characterization of the degree of parallel assembly in ribosomes because current biophysical techniques have difficulty resolving intermediates, especially with a large macromolecule ($>$500kDa) that requires high concentrations (100-1000nM) to assemble.

To quantitate 30S intermediates, Fluorescence Correlation Spectroscopy (FCS) was extended to create Fluorescence Triple Correlation Spectroscopy (F3CS). By correlating three signals, F3CS can study complex stoichiometric systems, kinetic processes and irreversible reactions. Theory, numerical correlation strategies, and experimental practices were established and a two-photon excitation, three-color detection microscope was built to perform the first F3CS measurements. The technique is implemented as a suite of software, Triple Correlation Toolbox, to enable triple correlation experiments with existing microscopes.

F3CS simultaneously identified intermediates both on and off the ribosome assembly pathways that were predicted by classic assembly maps. A weak energetic bias favors one assembly pathway over the others, but the bias energy is not strong enough to prevent the formation of intermediates off of the favored pathway. The interaction energies of ribosomal proteins S7, S9 and S19 are initially weak, which in principle reduces the potential for kinetic traps. F3CS has